Summary & Conclusions
Summary

It is apparent from the literature that breast cancer is a multigenic complex disease, there are multiple underlying biochemical mechanisms, no single gene or pathway will independently account for the onset, progression and severity of breast cancer in all individuals and no single biomarker with sufficient sensitivity and selectivity for all categories of breast cancer currently exists. Multiple pathways exist, each associated with a specific cellular morphology and/or tumor architecture (Korsching E et al 2002). Many clinical studies aimed at validating putative markers have produced contradictory reports. The profiles of different biological markers are indicative of clinical outcome (Daidone MG et al 1999) and the measurement of multiple biomarkers will allow us to biologically characterize individual tumors. To be clinically useful, the biomarkers to be measured must reveal the inherent biological characteristics of the breast cancer (Makris A 1997)

Numerous biomarkers associated with angiogenesis, cell cycle regulation, tumor cell proliferation, nuclear index and hormone receptors have been widely examined as independent markers and their prognostic and predictive significance is summarized in table-57

The emerging specialties of genomics, proteomics and metabolomics and the availability of high throughput screening strategies will help in the discovery of new pathways and in the better understanding of tumor
Significance of molecular markers in carcinoma of breast biology. DNA micro arrays, comparative genomic hybridization and single nucleotide polymorphism assay technologies may ultimately be deployed at the point-of-care, thereby enabling the simultaneous utilization of a large number of carefully selected biomarkers for a more objective prognostication. These “global” and “chip”- based technologies will also permit the widespread examination of large numbers of patients with in decreased time duration.

Table-57 Chromosomal location and functional role of biological markers in breast cancer

<table>
<thead>
<tr>
<th>Biological Markers</th>
<th>Location</th>
<th>Function</th>
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<tbody>
<tr>
<td><strong>Hormone related markers</strong></td>
<td></td>
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<tr>
<td>Estrogen Receptor</td>
<td>6q25.1</td>
<td>Hormone receptor. Serves as a ligand-activated transcription factor.</td>
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<tr>
<td>Progesterone Receptor</td>
<td>11q22.1</td>
<td>Hormone receptor. Induced by the activation of ER.</td>
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<td><strong>Cell cycle DNA related proteins</strong></td>
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<tr>
<td>HER2/Neu</td>
<td>17q11.2-q12</td>
<td>Proto-oncogene with tyrosine kinase activity. Regulates cell growth, survival and differentiation.</td>
</tr>
<tr>
<td>P53</td>
<td>17p13.1</td>
<td>Tumor suppressor, transcription factor, inhibition of abnormal DNA replication, facilitation of apoptosis.</td>
</tr>
<tr>
<td>Topoisomerase II-alpha</td>
<td>17q21.2</td>
<td>Control of DNA topology, DNA replication, cell cycle regulation, chromosome segregation.</td>
</tr>
<tr>
<td><strong>Cellular and proliferative activity</strong></td>
<td></td>
<td></td>
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<tr>
<td>Ki67 MIB-1</td>
<td>10q21</td>
<td>Proliferative marker, associated with nuclear grade and Mitotic Index</td>
</tr>
<tr>
<td>Bcl2</td>
<td>18q21.33</td>
<td>Proto-oncogene, inhibition of apoptosis</td>
</tr>
<tr>
<td>BAG-1</td>
<td>9p12</td>
<td>Multifunctional protein that regulates apoptosis, proliferation, transcription and metastasis.</td>
</tr>
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</table>

New evidences suggest that different cellular subgroups in the female breast give rise to subgroups of breast carcinomas with differing protein...
expression and cytogenetic alteration patterns that may be related to clinical behavior (Korsching E et al 2002), underscoring the need to use multiple markers. Until a single "perfect" marker is found, breast cancer prognosis will have to rely on the status of a panel of biomarkers targeting multiple pathways and mechanisms with validated prognostic significance to generate clinically useful information. We conclude that the present, accurate classification, prognostication and treatment of breast cancer can only be achieved by the use of a panel of selected biomarkers capable of detecting altered expression of proteins associated with critical pathways and mechanisms. Elucidating the biological nature of tumors by determining the status of a panel of markers that detect oncogene activation, tumor suppressor gene inactivation, increased angiogenic and proliferative potential, loss of epigenetic control and response to therapy would provide the much needed information to specifically tailor a patient's treatment regime. Thus, in strong support of significance of biomarkers the present study revealed following significant observations:

- Expression of Bcl2, BAG-1 and p53 was noted in 57%, 68% and 61% in tumors of breast carcinoma patients, respectively.
- A significant decrease in Bcl2 expression was noted with increase in tumor size, disease stage and tumor grade in younger patients.
- BAG-1 protein expression was found to be lower in advanced stage patients and histological grade III tumors.
- Higher incidence of p53 positivity was found in lymphnode positive patients and advanced stage patients.
In univariate survival analysis, Bcl2 overexpression was significantly associated with better overall survival in lymphnode negative patients.

In trivariate analysis patients with three marker positivity had reduced overall survival as compared to patients with one or two marker positivity followed by negative subgroup.

18%(29/165) patients express basal type (ER-PR-Her2-), 38%(63/165) Her2 +ve and ER/PR -ve and 16%(26/165) patients showed Luminal A(ER+PR+ Her2-) and 28%(47/165) patients showed Luminal B type(Her2+ER+/PR+).

Luminal B group showed higher incidence of Bcl2 and BAG-1 expression as compared to Luminal A type. No such difference was noted between Basal type (ER-PR-and Her2-) and ER-PR- and Her2+ breast tumors in relation to Bcl2 and BAG-1. Also no such observation was seen with p53 expression.

Topo II α expression was noted in 63% of the tumors among which 28% tumors exhibited high levels i.e. 2+ or 3+ staining intensity.

Topo II α expression was found more common with advancement of disease and in high grade tumors.

Topo II α expression was associated with BAG-1, Bcl2 and p53 over expression.

Topo II α expression was associated with high cellular proliferation and poor histological differentiation of the tumor.

Ki67 was noted in 44% (>20% nuclear positive) of the tumors. Ki67 expression when correlated with apoptotic markers BAG-1, Bcl2, p53.
and Topo II alpha, a significant positive correlation was noted with Bcl2 (P=0.018).

- Luminal B group showed higher incidence of Topo II alpha and ki67 expression as compared to Luminal A type. No such difference was noted between Basal type (ER-PR-and Her2-) and ER-PR- and Her2+ breast tumors in relation to Topo II alpha and ki67 expression.

- The size of the primer pairs showed p53 exon 5 (290 bp) and for exon 7 (210bp) positivity was noted, respectively.

- Immunohistochemical staining of p53 protein showed 31% p53 positivity which was lower than the incidence of exons 5 and 7.

- A higher incidence of p53 exon 7 was found in ER+ and PR+ tumors. Regarding p53 exon 5, a higher incidence was seen in older patients and ER+ tumors.

- Also, a positive correlation of p53 exon 5 and exon 7 was noted with p53 protein.

- A trend of higher expression of exon 5 was found in patients with higher HG (50%), early stage (41%) and smaller tumor size (75%). No such observation was found in case of Age, LN status and HP diagnosis.

- A trend of higher expression of exon 7 was found in patients of lower age group (14%), lower histological grade (16%), early stage (13%), smaller tumor size (40%) and with HP diagnosis of IDC (12%). No such observation was found in case of LN status of the patients.

- A trend of inverse correlation (P = 0.09) was found between tumor size and p53 exon 7.
A trend of higher expression of Bcl2 gene was found in patients of younger age group (14%), low HG (16%), early stage (13%), smaller tumor size (40%) and with HP diagnosis of IDC (12%). No such observation was found in case of LN status of the patients.

No correlation was found between ER, PR and Bcl2 gene

**Conclusions**

- Breast tumors with Bcl2 negativity, BAG-1 positivity and p53 positivity indicated patients with aggressive phenotype.
- Higher expression of Topoisomerase II alpha, proliferative marker correlated with the aggressive phenotype in breast cancer.
- ki67 expression in association with Bcl2 was suggestive of an aggressive phenotype. For breast cancer patients.
- p53 exons 5 and 7 expression was found to be associated with hormone dependent breast cancer.
- p53 protein expression was associated with hormone receptors negativity.
- P53 exon5 and 7 mutations found to be associated with p53 protein expression.

Thus, the present study on human tumor samples revealed strong clinical usefulness of the biomarkers including Bcl2, BAG-1, P53, Topoll alpha, Ki67 and Her2 Neu.

**Concluding remarks:**

Several recent developments emphasize the increasingly important role of immunohistochemistry. These genogenomic immunohistochemistry may
play a significant role in management of breast cancer patients as well as in search of proteins for targeted therapy. The recent concept of "Technician-free" automation of the IHC procedures and "Pathologist-free" microscopic image analysis technology for interpretation of high-throughput results will greatly change the current scenario. "Genogenic immunohistochemistry" heralds a new era in immunohistochemistry, and identification of the underlying molecular changes by immunohistochemistry is being used both for diagnosis and therapy. In addition to the prognosticators, the markers to monitor drug resistance include P-glycoprotein, the product of the MDR gene (multidrug resistant); N-cmyc and tumor suppressor genes such as p53; retinoblastoma susceptibility suppressor gene; putative suppressor genes-BRCA-1 gene, DNA repair genes (microinstability) are examples of genogenic immunohistochemistry. The genetic mutations such as loss of E-cadherin protein in lobular carcinoma of the breast, ALK overexpression to recognize the t(2,5) translocation in anaplastic large cell lymphoma, FLT-1 overexpression for the t (11;22) translocation of Peripheral nerve sheath tumor and Ewing sarcoma; Wilm's tumor-1 overexpression for the t(11;22) translocation of DSRTC are the newer examples of genogenic immunohistochemistry markers. Thus, the current study has established a strong ground for future developments in IHC for breast cancer.